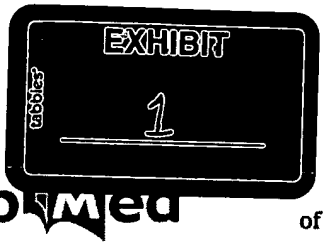

1: Hum Gene Ther 1997 Mar 1;8(4):403-10 Related Articles, NEW Books, LinkOut

Modulation of the specificity and activity of a cellular promoter in an adenoviral vector.

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Most gene therapy studies with recombinant adenoviruses employ viral promoters and lack tissue specificity. To determine whether a tissue-specific cellular promoter inserted into the adenoviral genome can direct the expression of a reporter gene in a tissue-specific manner, recombinant adenoviruses containing a nuclear lacZ gene driven by a human ventricular/slow muscle myosin light chain 1 promoter with and without a muscle creatine kinase enhancer were constructed. The ability of these viruses to express the reporter genes in infected myogenic and nonmyogenic cell lines was studied. Intramuscular injection of these viruses into mice showed that little or no reporter gene expression occurred in muscle fibers, although a relatively high level of lacZ gene expression was observed in surrounding connective tissue. Insertion of adenovirus sequences from the 5' inverted terminal repeat (ITR) region and/or the protein IX region into plasmids resulted in decreased reporter gene expression from myosin light chain 1 promoter in transfected C2C12 myotubes and 293 cells, as well as in injected muscles. These results suggested that negative elements are present in the adenoviral genome. This negative effect seems neither tissue nor species specific. Adenovirus cis-elements that may affect the specificity and activity of a cellular promoter are discussed.

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1: Gene Ther 2001 Feb;8(3):247-53

Creation of a new transgene cloning site near the right ITR of Ad5 results in reduced enhancer interference with tissue-specific and regulatable promoters.

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Tissue-specific transgene expression is a valuable research tool and is of great importance in delivering toxic gene products with adenovirus vectors to tumors. Limiting cytotoxic gene expression to the target cells is highly desirable. While a number of successful applications of tissue- and tumor-specific gene expression using Ad vectors has been reported, cloning of some promoters into Ad vectors resulted in modulation or loss of tissue specificity. This phenomenon is likely the result of the interaction of E1A enhancer (and possibly other Ad sequences) with the promoter cloned in the E1 region. We have compared performance parameters of prostate-specific and tet-regulatable promoters in plasmids containing the terminal repeat sequences of Ad5 with or without the E1A enhancer. Subsequently, adenoviral vectors were constructed containing identical expression units either in the E1 region or near the right ITR, and tested in several cell lines. Here, we report that promoters placed near the right ITR of Ad5 retain higher selectivity and lower background expression in both plasmid and adenovirus vectors. We confirm that the E1A enhancer can interfere with the desired activity of nearby promoters, and describe an alternative transgene insertion site for construction of Ad vectors.

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